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# Peritoneal dialysis solution biocompatibility: Definitions and evaluation strategies

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It is well established that materials employed for medical diagnostic and therapeutic purposes require biocompatibility testing before human use, as evidenced by the comprehensive and general guide to biocompatibility testing published in the International Standard ISO-10993 [1]. Similarly, in hemodialysis, biocompatibility profiling has become a prerequisite for any new membrane that is developed [2–5]. This requirement has been in response to the recognition that biocompatibility may play an important role both in acute intradialytic symptoms and chronic morbidities associated with hemodialysis therapy, such as susceptibility to infection, osteodystrophy, and amyloidosis. Today, biocompatibility profiling of peritoneal dialysis (PD) solutions has become an important feature of both conventional and new dialysis solution performance [6–11].

It is now over 20 years since the in vitro detrimental effects of commercial dialysis solution on phagocyte function were first described [12]. Since this time, there has been a plethora of reports describing a wide variety of approaches, techniques, and results of biocompatibility PD solution testing. The impetus for this proliferation of research is the growing belief that the first generation of commercially available PD solutions that have low pH, high lactate and glucose concentrations, are hyperosmolar, and contain glucose degradation products that may be causally associated with some of the complications of PD therapy [7, 8, 13–19]. Examples of the latter are pain upon infusion in the acute setting and loss of ultrafiltration in the long-term patient. The aim of this review is to discuss definitions of biocompatibility, the hierarchical testing schemes that are often selected for biocompatibility testing, how these schemes relate to contemporary models of structural and functional alterations of the peritoneal membrane, and the associated clinical consequences. Finally, an example of how the results of such a biocompatibility testing scheme helped to optimize the development of a new, physiologic pH and bicarbonate-lactate based solution, Physioneal.

**Key words:** bicarbonate, peritoneal dialysis, pH.



## DEFINITIONS OF BIOCOMPATIBILITY

In 1994, the Consensus Conference on Biocompatibility defined biocompatibility as "the ability of a material, device or system to perform without a clinically significant host response in a specific application." [20] This is an uncontroversial definition, but it is suggested that an enhancement may include the term a "clinically significant undesirable host response" assuming all host responses are not undesirable. This definition mandates human clinical evaluation and thus, for practical purposes during the research and development phases of new PD solutions or when more immediate insights into biocompatibility characteristics are required, alternative definitions have been proposed. In 1993, Holmes [9] defined PD solution biocompatibility as the biological effect that the solution exerts on the normal functioning of the tissues and cells of the peritoneum during both uninfected and infected states. This definition best lends itself to acute in vitro testing methods. Subsequently, Di Paolo [21] proposed that biocompatibility of PD solutions should include "the capacity to leave the anatomical and physiological characteristics of the peritoneum unchanged in time." Obviously, this definition is tailored to the use of animal models and clinical trials for biocompatibility evaluations, and does bring the important concept of the effect over time of any given formulation. Recent observations on structural changes in the peritoneal membrane over time on PD by the International Peritoneal Biopsy Registry, as described by Williams [22] in this supplement, clearly show that progressive alterations take years to manifest, emphasizing the challenge that lies in the interpretation of biocompatibility testing systems in which exposure times and conditions are often significantly different from those of the clinical situation. The latter limitation is particularly evident with in vitro systems, and to some extent even with animal models, although the development of long-term chronic dialysis models in recent years provides more relevant data [23–25].

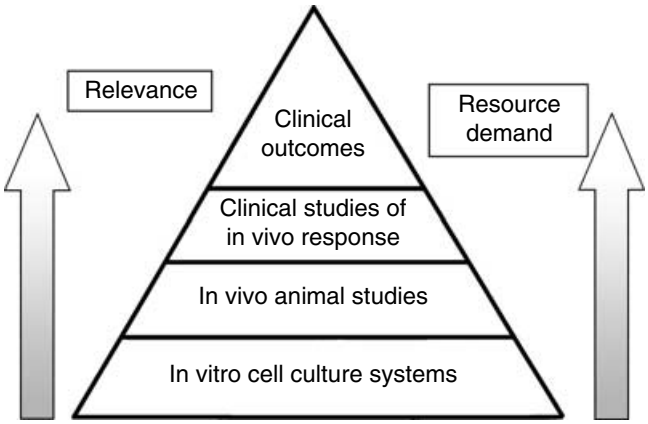


**Fig. 1. A hierarchical representation of biocompatibility testing.** The progression from in vitro to clinical studies usually results in a level of increasing clinical relevance, with a corresponding increase in the demand of expertise and resources.

## THE HIERARCHY OF BIOCOMPATIBILITY TESTING

Biocompatibility evaluations described in the scientific literature usually can be categorized into the following approaches: in vitro cell culture systems; in vivo animal models; clinical studies of in vivo response; or clinical outcome studies. Figure 1 describes a typical hierarchical representation of these approaches in terms of clinical relevance and degree of difficulty. In vitro assay systems often provide early indications of the biocompatibility performance of a dialysis solution, potentially providing input into the design of a new formulation, and importantly minimizing the need for future animal studies. As in vitro assay systems are almost uniformly much less technically and resource demanding than animal models, data from such studies constitute the preponderance of biocompatibility reports in the scientific literature. In this supplement, Hoff reviews extensively the wealth of in vitro assay systems that have been selected to date to assess PD solution biocompatibility. In vitro data can form the initial basis for concern over a seemingly bioincompatible performance of a dialysis solution. However, it should be always kept in mind that the frequent use of single cell types combined with culture conditions and solutions' exposure methods that do not closely mimic the in vivo experience limits the interpretation of such data. Similarly, the seemingly biocompatible performance of a new solution in vitro during its development cannot assure that in vivo or clinical indices will follow suit. The progression to animal models in order to provide in vivo biocompatibility performance usually constitutes the next phase of evaluation before human exposure (Fig. 1). Obviously, the benefit of in vivo exposure in which all peritoneal cell and tissue types and exposed simultaneously is evident. Nevertheless, the usefulness of animal data clearly depends upon how well the model recapitulates the human disease [21]. In the field of PD models, recent advances in the development of long-term models with minimal infection have permitted more meaningful solution biocompatibility testing [23, 25–27]. The advantages and disadvantages of various PD animal models are discussed by ter Wee in this supplement. However, certain circumstances exist, even with long-term animal models, which limit their interpretation. For example, the use of nonuremic animals is typical in this field.

The transition from in vitro and animal model preclinical testing to clinical evaluation now poses the opportunity to obtain early biocompatibility performance data using surrogate markers of peritoneal structure and function prior to the challenging task of performing human clinical outcome studies (Fig. 1). Topley et al describe typical clinical study designs in this supplement using in vivo markers of peritoneal membrane status, intraperitoneal inflammation, and peritoneal cell function. Fundamentally, clinical studies of in vivo response to a PD solution involve the assessment of changes in either effluent markers of inflammation, angiogenesis, and fibrosis present in patient dialysis effluent after timed in vivo dwell of the solution. Additionally, assessment of cell viability and function, usually macrophages isolated from effluent, can be included. The interpretation of the clinical relevance of such data depends upon the strength of the evidence supporting a pathogenic role for the mediator, cell, or cell function selected for study.

Despite the caveats associated with each stage of biocompatibility testing, it is proposed that information from studies higher in the hierarchy should supersede that obtained from other studies lower in the hierarchy if inconsistencies occur. Ultimately, the relevance of all biocompatibility testing will need to be established through a combination of registry data, both outcome and biopsy in content and, whenever feasible, using the gold standard of randomized controlled clinical outcome trials.

### Structural and functional correlates of biocompatibility testing

During the last five years, an increased understanding into the temporal structural changes of the peritoneal membrane, as described by Williams in this supplement, has also been accompanied by significant insights into the molecular and cellular pathobiology of peritoneal membrane dysfunction [28–37]. Combined, these observations have led several lead investigators in the field to construct hypothetical models that attempt to unify this information [38–40]. For the generalist, interpretation of biocompatibility studies in relation to these contemporary models can be challenging. For instance, in this supplement, there are reviews covering a variety of biocompatibility testing approaches of Physioneal, a new pH neutral bicarbonate-lactate based PD solution. The combination

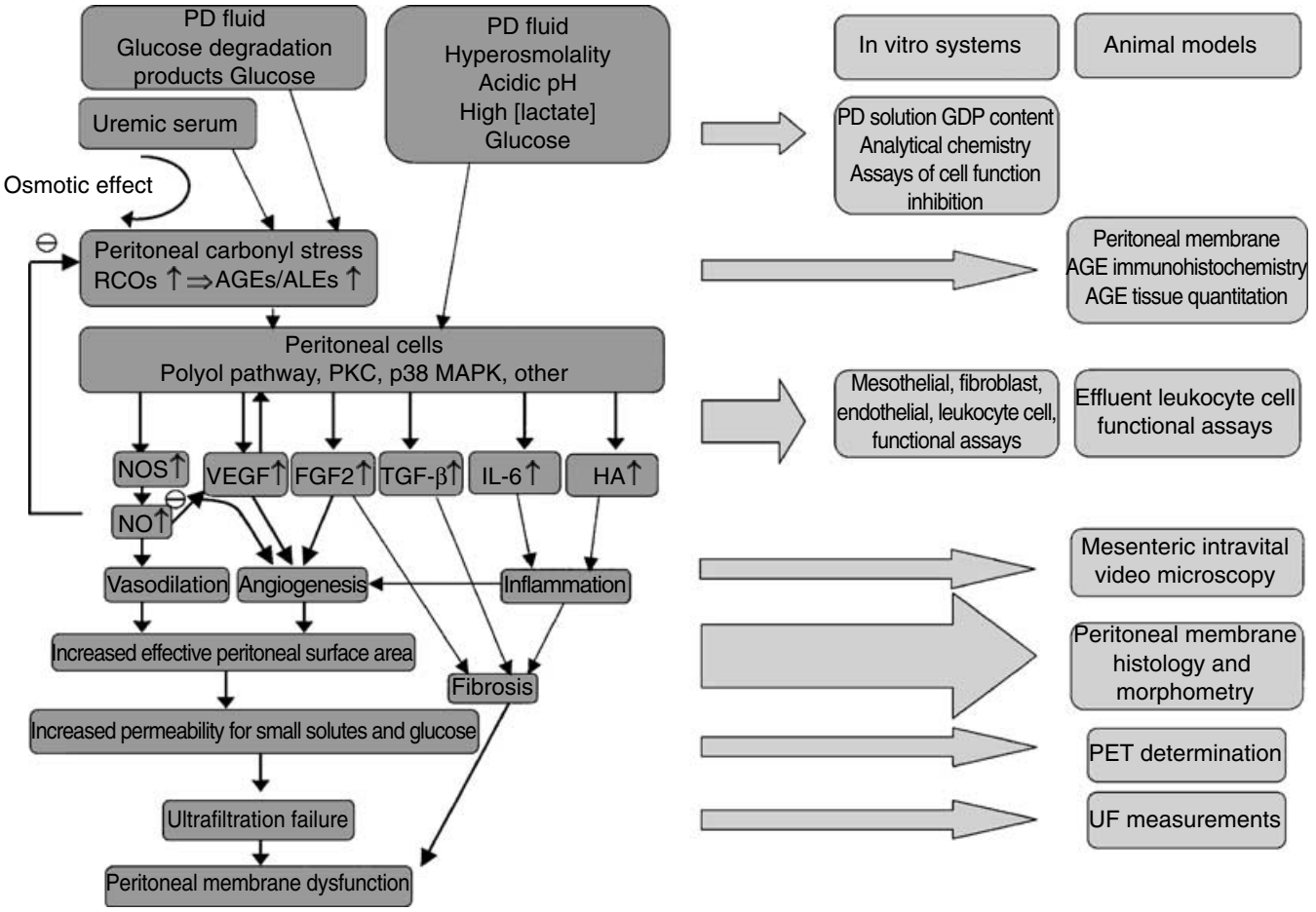


**Fig. 2. A contemporary model of peritoneal membrane dysfunction that provides detail of potential effectors and mediators of structural and function membrane alteration in the long-term patient.** The corresponding contribution of in vitro and animal model biocompatibility methods to each aspect of the model is provided. Modified from Miyata et al: *Kidney Int* 61:375–386, 2002.

of in vitro, animal, clinical studies of in vivo response, and clinical outcome studies, were employed to optimize the solution formulation during development and demonstrate benefit of the final formulation. How do such biocompatibility studies provide insight into contemporary models of membrane dysfunction? To illustrate, a recent and sophisticated hypothetical model proposed by Miyata et al [39] describing alterations of the peritoneal membrane in long-term patients who experience ultrafiltration failure has been selected and then modified to include biocompatibility studies and their corresponding utility (Figs. 2 and 3). Figure 2 illustrates where in vitro biocompatibility assays systems and animal model evaluations can provide insight into each of aspect of the hypothetical model. The roll and utility of clinical studies of in vivo response and clinical outcome studies to provide further insight into the effects of PD solutions on membrane dysfunction, as described by this model, are shown in Figure 3. It can be seen that each of the test methods chosen within a biocompatibility evaluation program will augment and compliment each other, ultimately providing a comprehensive understanding of the biocompatibility performance of any given solution formulation.

## Biocompatibility and new PD solution development: A case study

Having described an overview of biocompatibility evaluation strategies and hierarchical testing schemes, the following section provides a case study describing how a biocompatibility evaluation plan optimized the formulation development of a bicarbonate-based physiologic pH solution, Physioneal. Prior to the development of Physioneal, it had already been established that several factors were important in the biocompatibility performance of conventional PD solutions. These consisted of the low pH of the formulations, their lactate content, and the presence of glucose as the osmotic agent. Glucose contributes to bioincompatibility by virtue of its intrinsic metabolic, gene activating properties, its hyperosmolality at the concentrations employed, and because it is the source of glucose degradation products, some of which

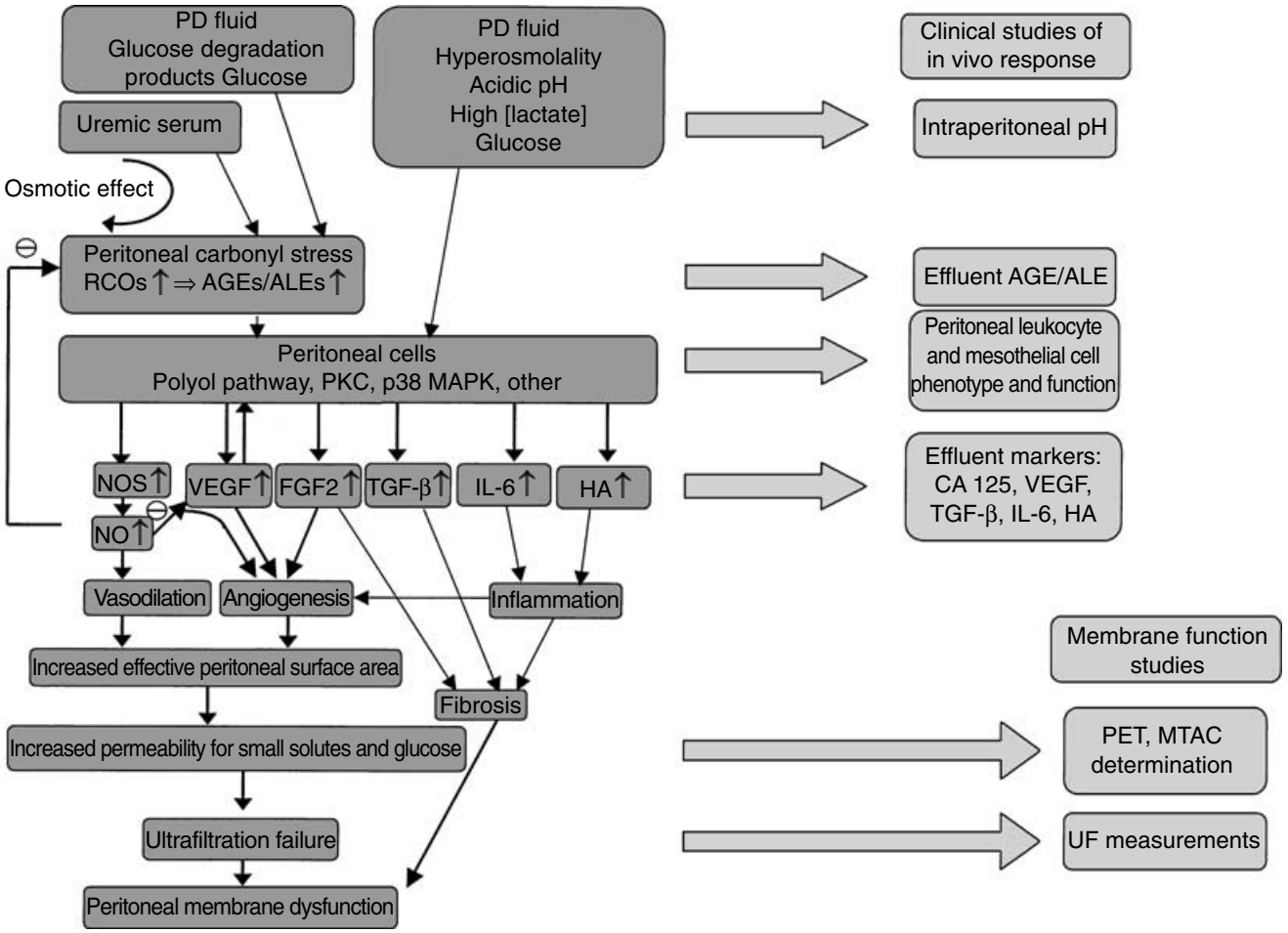


**Fig. 3. A contemporary model of peritoneal membrane dysfunction that provides detail of potential effectors and mediators of structural and function membrane alteration in the long-term patient.** The corresponding contribution of clinical studies of in vivo response and membrane function to each aspect of the contemporary model membrane dysfunction is provided. Modified from Miyata et al: *Kidney Int* 61:375–386, 2002.

may be cytotoxic and/or contribute to carbonyl stress. Hoff more extensively reviews these factors in this supplement. Reduction in total glucose exposure, glucose degradation products, hyperosmolality, and low pH can be partly addressed by the use of nonglucose-based solutions containing icodextrin or amino acids [41]. However, as these formulations cannot be used for all dialysis exchanges, until a safe and affordable alternative to glucose is identified, there remains the need for glucose-based solutions that have been optimized in their biocompatibility performance. Possible improvements in biocompatibility of glucose-based PD solutions therefore required the development of a PD solution that could be infused at a physiologic pH, contain an alternative buffer system, and provide a reduced glucose degradation product content.

In order to avoid caramelization of dextrose, glucose-based solutions have to be formulated at a pH of maximum 5.5. At pH values above this value excessive glucose degradation occurs, rendering the formulation unacceptable for human use. Container designs that permit separation of dextrose from lactate in a two-chambered bag allowed the adjustment of the pH of the lactate compartment to a higher level, and the glucose at a more acidic pH value. This design permits a pH level of 6.0 to 6.5 after mixing of the two chambers and a significant reduction in most glucose degradation products [42]. However, a pH of 7.4 still cannot be achieved with this improvement as lactate has most of its buffering capacity in a pH range of 3 to 5, with very minor buffering capacity at a pH of 6.0 to 6.5, resulting in unstable solutions in the upper pH range at a pH of 4 to 6.

From the discussion above, it is clear that the pH of the PD solution can be increased further by adding a solution buffer with a good buffering action in a pH range around 7.4. Bicarbonate is an ideal candidate for PD solutions as it provides the physiologic buffer of the extracellular space, while providing a solution buffering action in the correct pH range (5.5 to 7.5). Due to advancements in container and packaging technologies in recent years,

the ability to manufacture a stable bicarbonate-based PD solution became feasible. Early clinical development work resulted in the conclusion that bicarbonate-based PD solution would need concentrations of bicarbonate ion in the range of 35 to 40 mmol/L for adequate acid-base control. Although bicarbonate is regarded as the primary physiologic buffer, it was unclear if supraphysiologic concentrations of bicarbonate would be biocompatible [43–45]. Likewise, although it had been reported that exposure to high levels of carbon dioxide, as experienced during laparoscopic surgery, can decrease macrophage cytokine release, the effect of chronic exposure to lower, yet still supraphysiologic levels of $CO_2$ remained unclear [46]. The clinical impact of higher than physiologic bicarbonate and $pCO_2$ is unknown, but at least one study indicated that high pCO2 levels might have undesired side effects [47]. In addition, Schambye et al reported that a clear difference existed between buffer systems containing bicarbonate alone and mixtures of bicarbonate and other buffers using an in vitro assay of neutrophil migration [48–54]. Henderson and Martis therefore proposed a combination of 25 mmol/L bicarbonate plus 10 to 15 mmol/L lactate as an alternative formulation to pure bicarbonate for optimal biocompatibility [55]. This formulation was proposed to provide improved biocompatibility by proving a physiologic level of bicarbonate, $pCO_2$, and pH, while at the same time ensuring the highest level of safety. Because it was unknown whether the presence of supraphysiologic bicarbonate and $pCO_2$ present in a pure bicarbonate formulation would be disadvantageous relative to lower levels of lactate, but now at neutral pH, with the mixed-buffer formulation, a series of biocompatibility studies comparing the two formulations were conducted using in vitro, ex vivo, and clinical designs.

In vitro studies using a variety of cell types and assay conditions to compare pure bicarbonate and bicarbonate/lactate mixtures consistently revealed that both of these formulations were significantly more biocompatible than the conventional acidic lactate-based solutions with higher glucose degradation product content [56]. However, no differences between bicarbonate alone and bicarbonate-lactate formulations could be detected, suggesting equivalent biocompatibility. Due to the limitations of in vitro studies as described above, it was considered prudent to seek additional information using clinical indices of biocompatibility.

Associated with a Phase II clinical trial, biocompatibility studies were conducted to compare the in vivo response to bicarbonate alone and the mixed-buffer solution [57]. The design and results of these studies are extensively reviewed by Topley in this supplement. Briefly described, patients were exposed in a randomized crossover manner to either bicarbonate or bicarbonate/lactate solution after initial exposure to the control lactate solution, all within three consecutive days. After short intraperitoneal dwell periods permitting in vivo exposure, peritoneal macrophages were isolated from effluent and assessed for function using the release of both unstimulated and stimulated tumor necrosis factor α (TNFα). When solutions containing the lower glucose concentration of 1.36% were employed, macrophage function was significantly improved with both bicarbonate-based solutions versus the control solutions, interpreted by a higher release of stimulated TNFα, with no change in cytokine secretion when unstimulated. The response of macrophages exposed to the bicarbonate/lactate solution was significantly better than that seen with the bicarbonate alone formulation. Furthermore, a significant improvement in cell function was only seen with the bicarbonate/lactate mixture when 3.86% glucose-based solutions were employed. Invoking the hierarchy of biocompatibility relevance, the results of these Phase II clinical studies suggested that the bicarbonate/lactate formulation was to be superior to bicarbonate alone in biocompatibility performance, despite equivalence seen in earlier in vitro reports.

In an ideal world, this line of investigative research should have been pursued to its logical end—a multicenter, randomized, controlled study comparing each formulation with hard end points, such as ultrafiltration failure rates. However, the conduct of such a study is viewed as impractical due to logistical considerations, as insightfully described by Coles [58]. Nevertheless, pain upon infusion, although uncommon, was selected as a clinical end point for study in a randomized, double-blind, crossover study and proposed as index of biocompatibility [59]. The results of this study are reviewed by Lindholm et al in this supplement. Briefly, the effects of a 38 mmol/L bicarbonate solution, a bicarbonate/lactate solution (Physioneal), and standard solution on infusion pain were assessed using a verbal rating scale and the validated McGill Pain Questionnaire. Both of the test solutions resulted in a highly statistically significant reduction in inflow pain compared to the control lactate solution. Although differences were not large, for all pain variables assessed, the bicarbonate/lactate solution was reported to be more effective than the bicarbonate alone solution in alleviating pain. The combination of these clinical outcome observations with the in vivo clinical response studies led to the logical choice of the bicarbonate/lactate formulation as the final choice for further clinical development. The latter case study reveals the utility of a structured and comprehensive biocompatibility testing plan during new PD solution formulation development. In this particular case study, by following a biocompatibility test plan that transitioned from in vitro to clinical studies of in vivo response and clinical outcome studies, it was possible to identify an optimal formulation that could not have been achieved using theoretical considerations or in vitro studies alone.

## CONCLUSION

In recent years we have witnessed the development of a wide variety of approaches to the assessment of PD solution biocompatibility. These methodologies have grown to include an expanding array of in vitro, animal, and ex vivo techniques. Concurrently, the result of basic and clinical research into the molecular and cellular pathways involved in the structural and functional changes seen in some long-term patients has provided insight into the pathobiology of peritoneal membrane failure. These observations have permitted the development of several contemporary models of membrane dysfunction in the long-term patient. This review attempts to illustrate how biocompatibility testing schemes can provide valuable information on each component of these contemporary models. Furthermore, the use of a hierarchical and structured biocompatibility testing scheme to help direct and optimize the development of a novel bicarbonate/lactate based PD solution is provided.

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